which "shows incremental taxol and taxane production" is Fig. 5B. However, Applicants propose these amendments to the specification in the interest of clarity and definiteness. Applicants further note that identifying Fig. 5A and Fig. 5B as Panels A and B renders the identification clear, regardless of the relative placement of the panels.

Applicants are amending page 10, lines 10 and 13; page 12, line 13; page 25, line 23; page 27, line 7; and page 42, line 23 to insert quotation marks around certain words and phrases. Each of these words or phrases is preceded by the words "the term(s)," and is followed by an indication of what the word or phrase refers to, or how it is used. The meaning of the affected sentences is therefore readily apparent to one of skill in the art in the specification as-filed, and is not altered by these amendments. Applicants seek to make these amendments in the interest of clarity and readability.

Applicants seek to amend page 31, lines 18-19 to correct a typographical omission of words. It is readily apparent from reading the sentence that a typographical error was made. Likewise, the correct meaning, as given by the as-amended sentence, would be readily apparent to one skilled in the art in the context of the paragraph in which it appears, which addressees the production of taxanes by cells in culture. Applicants seek to make this amendment in the interest of clarity and definiteness.

Applicants are amending page 34, line 1 and page 45 line 19 to correct typographical errors. On page 34, the correct form is readily apparent to one of skill in the art in the context of the as-amended phrase "the transition from the growth medium [through] to production medium..." (page 33, line – page 34, line 1). On page 45, the error involves the misplacement of the word "growth" and the addition of an extra "into." It is readily apparent from reading the sentence that

a typographical error was made, and the correct form is readily apparent to one of skill in the art. Applicants seek to make these amendments in the interest of clarity and definiteness.

Page 43, lines 3, 6, and 15 and page 44, line 9 are amended to change "uM" to "μM" and to change "uL" to "μL". While the character "μ," denoting "micro" is often written as "u" because many typewriters and computers lack Greek characters, Applicants propose these amendments to the specification in order to ensure consistency and accuracy. That both "uM" and "μM" denote "micromolar" and that both "uL" and "μL" denote "microliter" is well-known to one of skill in the art. Further, as amended, the concentrations in the present application reflect the concentrations cited in parent application 08/653,036, which was incorporated by reference into the subject application as filed. Therefore, these amendments add no new matter to the disclosure as filed in the subject application.

Applicants seek to amend page 49, line 6 to correct a typographical error which incorrectly refers the reader to Table 8, rather than to Table 9. The correct Table is readily apparent to one of skill in the art upon reviewing the content of the paragraph spanning pages 48-49, Table 8, and Table 9. Both the paragraph that includes page 49, line 6 and Table 9 pertain to the manipulation of nutrient media to increase taxol productivities. In contrast, Table 8 presents data from experiments demonstrating the effects of chitosan-glutamate on production of taxanes.

Applicants seek to replace Tables 3, 4, and 8 to improve the alignment of the data and labels. The correct alignment is readily apparent upon reading the Tables. Additionally, the replacement Tables are identical to corresponding Tables 3, 4, and 8 of parent application, Serial No. 08/653,036, which is incorporated by reference in the subject application at page 1, lines 3-10.

Applicants are amending Table 7 to correct a typographical error. The error and the correct form are readily apparent upon reading Table 7, as the amounts shown in the columns of the Table are labeled with the unit "µg," and it is apparent that the legend to the Table should match the Table itself. Faced with the conflicting units in Table 7, one of skill in the art would assume that the "mg" unit was a typographical error for at least two reasons. First, a typist is likely to forget to type a "µ" because this character does not appear on the keyboard and requires several keystrokes or mouse clicks to produce. Likewise, computer software, or a printer, is likely to convert a "µ" to an "m," but the reverse would not occur. Second, the unit "µg" occurs consistently in the columns of the Table, so it is unlikely to be an error. In contrast, the unit "mg" occurs only once and could easily be a mistake.

Applicants seek to replace Tables 10 to 15 to correct typographical errors and to improve the alignment of the data and the labels. The typographical errors and the correct forms would be readily apparent to one of skill in the art reading the Table, especially in view of the concentrations and names of the same or similar compounds used in other tables. Were the errors corrected individually, there would be multiple replacements of "mM" with "µM" and of "a" with "α." Appearances of "-6-" in the middle of chemical names would also be corrected. Further, the replacement Tables are identical to corresponding Tables 10 to 15 of parent application, Serial No. 08/653,036, which is incorporated by reference in the subject application at page 1, lines 3-10.

Applicants are amending Table 16b to correct a typographical error. The as-filed Table reads "0,68 mM sodium phosphate." It would be readily apparent to one of skill in the art that the amount of sodium phosphate should be "0.68 mM." Further, the same amount of sodium phosphate appears elsewhere in Table 16a, at least in the composition of Feed F1.

Applicants propose to amend Table 17 to correct typographical errors in the text of footnote "a." Specifically, the amendment will replace multiple occurrences of "mM" with "μM" and to replace "1-naphthaleneacetic acid (NAA)" with "α-naphthaleneacetic acid (NAA)." Other than these changes, the text remains the same.

It would be readily apparent to one of skill in the art that the occurrences of "mM" which are sought to be replaced with "μM" should read "μM" because the same chemicals the concentrations of which these occurrences describe ("α-naphthaleneacetic acid, 3,4-methylenedioxynitrocinnamic acid, methyl jasmonate, and silver thiosulfate) are given in micromolar concentrations when they appear in other Tables. . (See, *e.g.*, amended Table 15, as-filed Table 16(a and b), and as-filed Table 18a). Likewise, it would be readily apparent to one of skill in the art that instances where "mM" is not sought to be replaced with "μM" should read "mM" because the same chemicals the concentrations of which these occurrences describe (glutamine) are given in millimolar concentrations when they appear in other Tables. . (See, *e.g.*, amended Table 15, as-filed Table 16(a and b), and as-filed Table 18a).

It would be readily apparent to one skilled in the art that "1-naphthaleneacetic acid (NAA)" should read "α-naphthaleneacetic acid (NAA)" because NAA had been used as an abbreviation for "α-naphthaleneacetic acid" in numerous instances in the Tables and in the text of the specification. (See, *e.g.*, amended Table 15, as-filed Table 16(a and b), and as-filed Table 18a).

Amendments to the Claims

Applicants seek to amend claim 1 to promote internal consistency within the claim and to explicitly recite that which was already implicit in the use of the phrase "nutrient media

comprises an enhancement agent," which necessarily means that other agents, such as another enhancement agent, may be included in the nutrient medium. Therefore, this amendment does not alter the scope of the claim. Further, the culture with more than one enhancement agent present is described, *inter alia*, at page 21, lines 24-25.

Applicants are amending claims 3 and 6 to maintain consistency with amended claim 1. These amendments do not alter the scope of the claims.

Applicants propose to amend claim 7 to clarify it. Claim 7 is amended to remove the express recitation of alkyl esters of jasmonic acid and dihydrojasmonic acid. This amendment is made in the recognition that said alkyl esters are already covered by claim 7, as claim 7 depends from claim 1, which includes alkyl esters of jasmonate-related compounds. Consequently, claim 7 encompasses alkyl esters of jasmonic acid and alkyl esters of dihydrojasmonic acid, without explicitly so reciting. Therefore, this amendment does not alter the scope of the claim set.

Claim 7 is also amended to remove the express recitation of "related derivatives and analogs" of jasmonic acid and of dihydrojasmonic acid. This amendment is made in the recognition that "jasmonate-related compound" as recited in claims 1 and 7, includes both the compounds recited in the specification, for example at page 28, lines 18-25, and compounds which are analogs of or otherwise related to these compounds. See, page 28, line 25. Consequently, compounds which are related to or analogs of jasmonic acid are covered by claim 1. Therefore, the scope of the claim set is not changed by this amendment.

Applicants propose to amend claim 9 to clarify antecedent basis in claim 8, Applicants seek to amend claim 12 to clarify antecedent basis in claim 11, and Applicants are amending claims 39 and 40 to clarify antecedent basis in claim 1. Applicants propose to amend

claim 41 to correct a typographical error in syntax. The correct form is readily apparent to one skilled in the art. None of these amendments alter the scope of the claims.

Applicants seek to amend claim 42 to clarify that the second medium has a composition that is different from the composition of the first medium. The scope of claim 42 is not altered by this amendment. Additionally, the amendment is supported, *inter alia*, on page 16, line 22 to page 17, line 11.

Applicants propose to amend claim 49 in the interest of consistency of claim language between claims 49 and 54. Specifically, Applicants are replacing the phrase "exchanging nutrient medium at least once during taxane production" with the phrase "periodic nutrient medium exchange." The meaning of these two phrases is identical, and therefore the substitution of one for the other does not change the scope of the claim.

Applicants propose to amend claim 46 in the interest of clarity and definiteness and to reflect the scope of the claim as originally filed. Applicants propose to amend claim 66 in the interest of clarity and definiteness. These amendments do not substantively change the claims as filed (claim 46) or as added by the Preliminary Amendment filed on 15 September, 1998 (claim 66).

Applicants propose to amend claim 69 to correct a typographical error. This error and its correction are readily apparent in view of the inclusion of the antecedent bases for "said amino acids" and "said polyamines" in claim 68, and in view of the lack of a claim 71 prior to the current responsive amendment. This amendment does not alter the scope of the claim.

New Claims

Applicants are adding new claims 71 and 72 to specifically claim additional aspects of the present invention, which aspects are fully supported in the specification as filed. In particular, claim 71 the inclusion of silver ions, silver complexes, and silver-containing compounds at a concentration of from is 0.01 μM to 0.1 μM is supported in the specification as filed, at least at page 23, lines 13-15, which states that "[w]hen silver is incorporated in the medium, it will be added at a concentration of at least 10 nM [= 0.01 μM], preferably 100 nM [= 0.1 μM], more preferably 1μM, and typically at 10μM." The addition of β-phenylalanine in the nutrient media is supported in the specification as filed, at least at page 25, lines 22-27, where it is taught that biosynthetic precursors may be added to the nutrient media and that β-phenylalanine is a suitable precursor.

Applicants do not believe that either of new claims 71 nor 72 correspond to any claims in United States Patent 5,637,484 to Yukimune et al. All of the claims of Yukimune require the presence of jasmonic acids. In contrast, claim 2 of the present application, from which new claim 71 depends, does not require the presence of jasmonic acids. Hence, neither claim 2 nor claim 71 of the present application correspond to any claim of Yukimune. Nowhere does Yukimune claim the use of any biosynthetic precursor in general, nor does Yukimune claim the use of β-phenylalanine in particular. Hence, new claim 72 does not correspond to any claim of Yukimune.

Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1-3, 6-21, and 24-70 are pending in this application. Claims 1 and 9 stand rejected under 35 U.S.C. § 112. Claim 1 stands rejected under 35 U.S.C. § 112, first paragraph, as being confusing. Claim 9 stands rejected under 35 U.S.C. § 112, first paragraph, as containing

New Claims

Applicants are adding new claims 71 and 72 to specifically claim additional aspects of the present invention, which aspects are fully supported in the specification as filed. In particular, claim 71 the inclusion of silver ions, silver complexes, and silver-containing compounds at a concentration of from is 0.01 μM to 0.1 μM is supported in the specification as filed, at least at page 23, lines 13-15, which states that "[w]hen silver is incorporated in the medium, it will be added at a concentration of at least 10 nM [= 0.01 μM], preferably 100 nM [= 0.1 μM], more preferably 1μM, and typically at 10μM." The addition of β-phenylalanine in the nutrient media is supported in the specification as filed, at least at page 25, lines 22-27, where it is taught that biosynthetic precursors may be added to the nutrient media and that β-phenylalanine is a suitable precursor.

Applicants do not believe that either of new claims 71 nor 72 correspond to any claims in United States Patent 5,637,484 to Yukimune et al. All of the claims of Yukimune require the presence of jasmonic acids. In contrast, claim 2 of the present application, from which new claim 71 depends, does not require the presence of jasmonic acids. Hence, neither claim 2 nor claim 71 of the present application correspond to any claim of Yukimune. Nowhere does Yukimune claim the use of any biosynthetic precursor in general, nor does Yukimune claim the use of β-phenylalanine in particular. Hence, new claim 72 does not correspond to any claim of Yukimune.

Rejection Under 35 U.S.C. §112, First Paragraph

Claims 1-3, 6-21, and 24-70 are pending in this application. Claims 1 and 9 stand rejected under 35 U.S.C. § 112. Claim 1 stands rejected under 35 U.S.C. § 112, first paragraph, as being confusing. Claim 9 stands rejected under 35 U.S.C. § 112, first paragraph, as containing

subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse these rejections.

Claim 1 stands rejected under 35 U.S.C. § 112, first paragraph, as being confusing. Applicants note that enhancement agents and their function are discussed at length in the specification as-filed. Enhancement agents are substances "which relieve one or more of the rate-limiting steps in taxane biosynthesis." (See specification, page 21, lines 6-10). Classes of enhancement agents are listed at, e.g., page 21, line 27 to page 22, line 2. Additionally, classes of enhancement agents are described at length in the text following this list, at least on pages 22 to 31 Further, an extensive list of exemplary enhancement agents is provided as Table 1. Hence, the function of enhancement agents is readily ascertainable from the specification, so that one reading claim 1 in the context of the specification would not find claim 1 to be confusing or unclear. Applicants respectfully request that this rejection be withdrawn.

Claim 9 stands rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. Applicants respectfully traverse this rejection.

As-filed, claim 9 of the present application indicates that, in a method for producing high yields of taxol and taxanes in cell cultures of *Taxus* species, the nutrient media comprises an alkyl ester of jasmonic acid, "wherein the alkyl group esterified to jasmonic acid has from one to six carbon atoms." On September 15, 1998, a Preliminary Amendment was made to claim 9 to claim the use of alkyl esters of jasmonic acid in which the alkyl group esterified to jasmonic acid has from

one to four carbon atoms. Page 28 was also amended to insert a statement indicating that alkyl esters of jasmonic acid which have from one to four carbon atoms in the alkyl group esterified to jasmonic acid are preferably used in the present invention.

Support for both the amended claim and the inserted statement is found, *inter alia*, in claim 4 of parent application, Serial No. 08/653,036, which is incorporated by reference in the subject application at page 1, lines 3-10. As-filed claim 4 of application Serial No. 08/653,036 claims a method for producing high yields of taxol and taxanes in cell cultures of *Taxus* species, wherein the nutrient media comprises an alkyl ester of jasmonic acid, "wherein the alkyl group esterified to jasmonic acid has from one to four carbon atoms." This claim, at least, clearly conveys to one skilled in the art that Applicants had possession of the invention claimed in as-amended claim 9 at the time that the parent application was filed, and consequently, at the time the subject application was filed as well.

Additional support for the claim and statement is found, *inter alia*, on page 28, lines 18-20 of the present specification as-filed, which indicates that "Jasmonate-related compounds include jasmonic acid and its alkyl esters, such as methyl jasmonate, ethyl jasmonate, propyl jasmonate, butyl jasmonate ...." One of skill in the art would readily recognize that an alternative way of specifying the same compounds would be to say, *e.g.*, "jasmonic acid and its alkyl esters, where the alkyl groups have from one to four carbon atoms."

Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 1-3, 6-21, and 24-70 are pending in this application. Claims 1-3, 7, 9, 12, 21, and 24-70 stand rejected under is rejected under 35 U.S.C. § 112, second paragraph, as being

indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as their invention. Applicants respectfully traverse these rejections.

Claim 7 stands rejected under 35 U.S.C. § 112, second paragraph, as encompassing an improper Markush grouping and being confusing in that the antecedent basis for "related derivatives and analogs" is unclear. Applicants' proposed amendment to claim 7 will remove the questioned language. Applicants therefore respectfully submit that this rejection is rendered moot.

Claim 9 stands rejected under 35 U.S.C. § 112, second paragraph, as indefinite for lack of antecedent basis. Specifically, the phrase "the alkyl group esterified to jasmonic acid" is said to fail to find proper antecedent basis in claim 8. Applicants are amending claim 9 to clarify antecedent basis. Applicants therefore assert that this rejection is rendered moot.

Claim 12 stands rejected under 35 U.S.C. § 112, second paragraph, as failing to find proper antecedent basis for "the heavy metal." Applicants are amending claim 12 to clarify antecedent basis. Applicants therefore assert that this rejection is rendered moot.

Claim 32 stands rejected under 35 U.S.C. § 112, second paragraph, as being confusing because the meaning of the phrase "auxin-related growth regulator" is unclear. Applicants note that the term "auxin-related growth regulator" is defined in the specification as-filed, at least on page 23, lines 17-18, as including "auxins, compounds with auxin-like activity, and auxin antagonists." Further, many examples of auxin-related growth regulators are provided in the specification, at least at page 23, line 20 to page 25, line 13. Applicants respectfully submit that the intended meaning of "auxin-related growth regulator" is unambiguous and that claim 32 is therefore not confusing. Applicants respectfully request that this rejection be withdrawn.

Claims 39 and 40 stand rejected under 35 U.S.C. § 112, second paragraph, for failing to find proper antecedent basis for "uninduced suspension culture." Applicants traverse these rejections. However, in the interest of facilitating prosecution, Applicants are amending claims 39 and 40 to clarify antecedent basis. Claims 39 and 40 as-amended clearly find antecedent basis in claim 1, as they now refer to culture without the addition of the enhancement agents specified in claim 1. Applicants therefore assert that these rejections are rendered moot.

Claim 41 stands rejected under 35 U.S.C. § 112, second paragraph, as being confusing in the recitation of "the said." Applicants are amending claim 41 to remove "the." Applicants therefore assert that this rejection is rendered moot.

Claim 42 stands rejected under 35 U.S.C. § 112, second paragraph, as being incomplete and confusing. Applicants are amending claim 42 to clarify that the second medium has a composition that is different from that of the first medium. Applicants respectfully submit that this rejection is rendered moot.

Claim 43 is rejected under 35 U.S.C. § 112, second paragraph, as vague and indefinite because the amounts intended by "lower" and "higher" are allegedly undefined. Applicants respectfully submit that a comparative term, such as "lower" and "higher," that compares the concentration of a specific component in two specifically recited media is not indefinite. In the case of claim 43, the second medium contains a concentration of nitrate that is lower than the concentration of nitrate in the first medium; and the second medium contains a concentration of saccharide that is higher than the concentration of saccharide in the first medium. Applicants respectfully request that this rejection be withdrawn.

Claims 43-48 stand rejected under 35 U.S.C. § 112, second paragraph, as failing to delineate the nature of "saccharide." Applicant notes that claim 44 does not contain the term "saccharide." Applicants have chosen to use the word "saccharide" in claim 66 in order to match the language of copied claims 50 and 61 of United States Patent 5,637,484 to Yukimune et al. Consequently, Applicants have used the word "saccharide" in claims 43 and 45-48 to promote internal consistency within the claims.

Applicants note that a saccharide is "A sugar or polymer of sugars linked by glycosidic (acetal) bonds [cross-references omitted]." Glick DM, *Glossary of Biochemistry and Molecular Biology*, Raven Press, 1990. The present patent application contains numerous exemplary descriptions regarding the nature of the saccharides that may be used in the methods of the present invention. For example, the specification indicates that "one or more nutrient media used in the cultivation of the cells may include maltose, sucrose, glucose and/or fructose as a carbon source." (See specification, page 8, lines 5-6). Similarly, page 15 teaches that lactose, galactose, raffinose, mannose, cellobiose, arabinose, xylose, sorbitol, glucose, fructose, sucrose, and maltose are preferred carbon sources for the cell cultures of the present invention. (See, specification, page 15, lines 3-5). Given this wide variety of examples, which include mono- and oligo-saccharides, Applicants submit that the skilled artisan can easily select a suitable sugar, or "saccharide," for use in the cultures of the present invention. Therefore, Applicants respectfully request that this rejection be withdrawn.

Claims 49 and 54 stand rejected under 35 U.S.C. § 112, second paragraph, as unclear. Applicants are amending claim 49, so that both claims recite "periodic nutrient medium exchange." Applicants respectfully assert that the nature of the process of "periodic nutrient medium exchange" is described in the specification sufficiently to allow one skilled in the art to perform this process.

For example, "medium exchange," as used in this application, is defined on page 35, lines 15-17. Additionally, guidance regarding the performance of periodic medium exchange is provided by Example 14 of the specification. (See, specification, page 55, line 20 to page 56, line 19.

Further, "medium exchange" is an art-recognized term. If the Examiner maintains that the concept of medium exchange is not clear in the art, Applicants respectfully request the Examiner to provide evidence for this assertion in the form of an affidavit under MPEP 2144.03. It is clear that "medium" is short-hand for "nutrient medium" in the context of this application, and in the context of the art of cell culture generally. "Periodic" carries its common meaning of "from time to time," or "at regular intervals." It is within the abilities of one skilled in the art of cell culture to determine when to exchange the medium.

Claim 66 stands rejected under 35 U.S.C. § 112, second paragraph, as unclear. Applicants are amending claim 66 to clarify it. Consequently, Applicants assert that this rejection is rendered moot.

Claim 69 is rejected under 35 U.S.C. § 112, second paragraph, as incomplete as depending on claim 71, which is not of record. Applicants are amending claim 69 to depend from claim 68, which is pending, and which provides proper antecedent basis for claim 69. Applicants therefore assert that this rejection is rendered moot.

No new matter is added by the amendments, and they are fully supported by application as filed and by the parent application, Serial No. 08/653,036. Therefore, Applicants respectfully request entry of these amendments.

Applicants believe that this application is now in condition for allowance, and such disposition is earnestly solicited. If the Examiner believes that prosecution might be furthered by discussing the application with Applicants' representatives, in person or by telephone, we would welcome the opportunity to do so.

Respectfully submitted,
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Table 3.

Preferred conditions for callus proliferation for various Taxus species. The ingredients in the basal media are listed in Table 2.

Species	Basal Medium (Table 2)	Growth Regulators*			
		Auxin		Cytokinin	
		Type	Conc (M)	Type	Conc(M)
T. brevifolia	F	P	5×10^{-6}	2iP	10^{-7}
	D	P	5×10^{-6}	BA	10^{-8}
T. canadensis	H	P	5×10^{-6}	K	10^{-7}
	D	P	5×10^{-6}	BA	10^{-8}
T. chinensis	D	P	5×10^{-6}	BA	10^{-8}
	A	N	5×10^{-6}	BA	10^{-8}
T. globosa	D	P	5×10^{-6}	BA	10^{-8}
T. floridana	D	P	5×10^{-6}	BA	10^{-8}
T. baccata	D	P	5×10^{-6}	BA	10^{-8}
T. cuspidata	D	P	5×10^{-6}	BA	10^{-8}
T. media	D	P	5×10^{-6}	BA	10^{-8}
T. wallichiana	D	P	5×10^{-6}	BA	10^{-8}

*Abbreviations: Picloram (P), Naphthalene acetic acid (N), Benzyladenine (BA), Dimethyl allylamino purine (2iP), Kinetin (K)

Table 4.

Typical growth characteristics of Taxus sp. suspension cultures

Species	Dry Weight Doubling Time	Fresh Weight Doubling Time	Dry Wt. Density	Fresh Wt. Density
T. brevifolia	2.0 days	3.5 days	20 g/L	400 g/L
T. baccata	2.0	6.0	15	220
T. chinensis	2.5	4.5	20	285
T. canadensis	nd*	8.5	13	260

*not yet determined

TABLE 7.

Effect of Standard GroLux light treatment on taxol and taxane content in 10-day old cultures of *Taxus chinensis* line K-1 cultivated in Medium A. Amounts shown are expressed as μg extracted from 20 ml of suspension. Cell growth was identical in both treatments (164 mg dry weight per flask).

	Light	Dark
Total taxol: cells and medium:	8.8 μg	3.13 μg
Extracellular taxol:	76.40%	56.20%
Total taxanes cells and medium:	61.55 μg	62.17 μg
Extracellular taxanes:	89%	84%

C6

TABLE 8

Comparison of chitosan-glutamate treated to non-elicited suspensions of *Taxus chinensis* line K-1 after 15 days cultivation in medium C. Taxane levels reported are from cells and medium combined. % extra refers to the percentage of extracellular

	CONTROL			ELICITOR		
	Cell density 10.1 g/L			Cell density 14.2 gm/l		
	Cell viability 70-80% viable			Cell viability 75-80% viable		
Taxanes	% dry wt	mg/L	% Extra	% dry wt	mg/L	% Extra
Taxol	0.054	5.4	7.2	0.098	13.9	85.0
Baccatin III	0.057	5.8	69.9	0.055	7.8	76.6
7-Xylosyl-10-deacetyltaxol	0.040	4.0	63.0	0.048	6.9	77.0
10-deacetyltaxol	0.0004	0.4	71.1	0.0	1.0	75.3
Cephalomannine						
10-deacetylbaccatin III						
10-deacetyl-7-epitaxol	0.054	5.4	74.2	0.076	10.8	85.7
7-Epitaxol	0.009	0.9	74.6	0.009	1.3	86.2
Unknown Taxanes	0.203	20.5	79.7	0.240	34.1	90.2
Total Taxanes:	0.421	42.4		0.533	75.8	

Table10
Enhancement of Taxane Biosynthesis in *Taxus chinensis* cell line KS1A by Silver

Silver Compound	Dose (μM)	mg/L extracellular product**		
		Baccatin III	Taxol	Total Taxanes
Culture Medium only*		16	5	21
Silver thiosulfate	50	71	15	86
Silver phosphate	100	48	7	55
Silver benzoate	20	40	7	47
Silver sulfate	20	61	7	68
Toluenesulfonic acid silver salt	20	39	6	45
Silver chloride	10	22	18	40
Silver oxide	50	43	18	61
Silver acetate	10	52	10	62
Silver nitrate	20	63	6	69

* The culture medium was Medium N from Table 2, with the addition of the following growth regulators: 10 μM α-naphthaleneacetic acid, and 1 μM thidiazuron
** All samples were taken after 14 days of incubation.

Table 11.
Enhancement of Taxol and Taxane Biosynthesis by Silver in several *Taxus chinensis* cell lines. The titers represent levels measured in the whole broth, i.e., in the cells and in the extracellular medium.

Cell Culture	Silver[a] Concentration	Culture Medium	Duration (days)	Baccatin III mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
SS6A-1224	0	I[b]	30	10	48	23	81
SS6A-1224	50 μM	I	30	172	86	126	384
SS122-13	0	II[c]	14	2	21	10	33
SS122-13	50 μM	II	14	12	103	60	173
SS122-42	0	II	14	3	80	26	109
SS122-42	50 μM	II	14	4	146	38	188

[a] Added as silver thiosulfate
[b] The culture medium is Medium N from Table 2, with the addition of the growth regulator, α-naphthaleneacetic acid at a concentration of 10 μM.
[c] The culture medium is Medium N from Table 2, with the addition of the growth regulator, α-naphthaleneacetic acid at a concentration of 10 μM and thidiazuron at a concentration of 1 μM.

Table 12
Enhancement of Taxol and Taxane Biosynthesis by Jasmonic acid and its methyl ester.Taxane titers were measured in the whole broth after 14 days of cultivation. The culture medium was Medium N from Table 2, with the additional presence of the growth regulator, α-naphthaleneacetic acid at a concentration of 10μM.

Cell Culture	Jasmonate Concentration	Baccatin III mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
SS122-42	0	3	80	26	109
SS122-42	200 μM JMA	4	120	87	211
SS122-42	89 μM MJS	3	121	109	233
SS122-13	0	2	21	10	33
SS122-13	89 μM MJS	9	73	63	124

C9

[a] JMA denotes the free acid, and MJS denotes methyl jasmonate

Table 13
Enhancement of Taxol and Taxane Biosynthesis by 3,4-methylenedioxynitrocinnamic acid (MDNA). Taxane levels were measured in the whole broth after 14 days of cultivation. The cell line used was *Taxus chinensis* SS122-42.

MDNA Concentration	Culture Medium[a]	Baccatin III mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
0	I	3	80	26	109
50 μM	I	5	163	45	213
50 μM	II	34	311	89	434

[a] The culture medium I refers to Medium N from Table 2, with the additional presence of the growth regulator, α-naphthaleneacetic acid at a concentration of 10μM. The culture medium II is identical to Culture medium I, with the additional presence of 50 μM silver thiosulfate.

Table 14
Enhancement of Taxol and taxanes in cell cultures of *Taxus chinensis* using various combinations of enhancement agents. All taxane concentrations are expressed as whole broth titers (i.e., concentration in cells and medium combined), and values were obtained after 11 days of incubation.

Cell Culture	Culture Medium[a]	Baccatin mg/L	Taxol mg/L	Other Taxanes mg/L	Total Taxanes (mg/L)
SS64-412	I	41	464	101	606
SS64-561	II	590	182	388	1160
SS64-571	III	596	158	261	1015
SS124-77	IV	72	39	576	687
SS122-29	V	18	306	152	476
SS85-26	VI	586	100	416	1102

[a] The culture medium for all combinations was Medium N in Table 2. Culture Medium I contained, in addition to Medium N, 10 μM α-naphthaleneacetic acid (NAA), 3 μM thidiazuron (TDZ), 50 μM 3,4-methylenedioxynitrocinnamic acid (MDNA), 89 μM methyl jasmonate (MJS), and 50 μM silver thiosulfate (SLTS). Culture Medium II contained, in addition Medium N, 10 μM NAA, 1 μM TDZ, 50 μM MDNA, 89 μM MJS, 10 μM SLTS, and an additional 98.5 mg/L sodium phosphate (monobasic). Culture medium III contained, in addition to Medium N, 10 μM indolebutyric acid, 3 μM TDZ, 30 μM 3,4-methylenedioxycinnamic acid, 89 μM MJS, and 50 μM SLTS. Culture medium IV contained, in addition to Medium N, 10 μM NAA, 89 μM MJS, 100 μM SLTS, and 5 mM glutamine. Culture medium V contained, in addition to Medium N, 10 μM NAA, 89 μM MJS, and 50 μM SLTS. Culture medium VI contained, in addition to Medium N , 10 μM NAA, 1 μM TDZ, 50 μM MDNA, 18 μM MJS, 50 μM SLTS, and 5 mM glutamine.

Table 15
Enhancement of Taxane Production by Medium Exchange.

Cell Line	Culture Medium[a]	Type of Operation[b]	Duration (days)	Product[c]	Production Level[d] (mg/L)	Ave. Volumetric Productivity[e] (mg/L/day)
Paella	I	Batch	11	Taxol	185	13
Paella	I	Medium exchange	20	Taxol	265	17
SS29-3A5	II	Batch	14	Baccatin III	260	18
SS29-3A5	II	Medium exchange	28	Baccatin III	580	21
SS29-3A5	II	Batch	22	10-deacetyl-baccatin III	300	14
SS29-3A5	II	Medium exchange	28	10-deacetyl-baccatin III	400	14
SS45-146	III	Batch	11	Total Taxanes	700	64
SS45-146	III	Medium exchange	28	Total Taxanes	2500	89

[a] The culture medium for these culture conditions was Medium N in Table 2. Culture medium I included, in addition to Medium N, 10 μM α-naphthaleneacetic acid (NAA), 1 μM thidiazuron (TDZ), 50 μM 3,4-methylenedioxynitro-cinnamic acid (MDNA), 18 μM methyl jasmonate (MJS), and 10 μM silver thiosulfate (SLTS). Culture medium II included, in addition to Medium N, 10 μM NAA, 1 μM TDZ, 50 μM MDNA, 89 μM MJS,10 μM SLTS, and 5 mM glutamic acid (monopotassium salt). Culture medium III included, in addition to Medium N, 10 μM NAA, 2.5 μM zeatin, 30 μM MDNA, 89 μM MJS, and 50 μM SLTS.

[b] Repeated enhancement was achieved by medium exchange, as described in Example 14.

[c] The predominant product produced by a given cell line under the specified culture medium is listed; taxanes other than the predominant product were also produced in each case, except for cell line SS45-146, for which total taxane production is listed.

[d] The production levels for batch cultivation refer to extracellular concentrations, i.e., the amount of taxane measured in the extracellular medium divided by the volume of the extracellular medium. For repeated enhancement by medium exchange, the production level refers to the total amount of taxane measured in the extracelluar medium after each medium exchange, divided by the suspension volume.

[e] The average volumetric productivity is one indicator of biosynthetic capability; it is defined as the total product divided by the suspension volume, and futher divided by the duration of the incubation.

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TABLE 16.b.

Details of fed-batch operation described in Table 16.a.

Feed solution	Composition	Feed rate (mL/L/day)	Start of feed (day)	Duration of feed (days)
F1	25% (weight/volume) (w/v) fructose, 25 mM glutamine, 50µM NAA, 250 µM SLTS, 89 µM MJS, 1.48 mM calcium chloride, 0.63 mM magnesium sulfate, 0.68 mM sodium phosphate (monobasic).	10	7	17
F2	F1, 75 mM α-phenylalanine, 25 mM β-phenylalanine	10	7	17
F3	25% (w/v) fructose, 150 mM α-phenylalanine, 25 mM β-phenylalanine	10	6	25
F4	50% (w/v) glucose, 5.92 mM calcium chloride, 2.52 mM magnesium sulfate, 2.72 mM sodium phosphate (monobasic), 500 µM SLTS, 10 µM TDZ, 100 µM NAA, 150 mM α-phenylalanine, 50 mM β-phenylalanine	5	9	22
F5	contained 50% (w/v) glucose, 100 µM NAA, 10 µM TDZ, 500 µM SLTS, 89 µM MJS, 0.68 mM sodium phosphate (monobasic), 50 mM α-phenylalanine	5	12	9